ANNUAL PROGRESS REPORT

PROJECT TITLE: Molecular Mechanisms of Metabolic Suppression: Protein synthesis and mitochondrial respiration in a hibernating ground squirrel model.

PRINCIPAL INVESTIGATOR: Dr. Bert B. Boyer (e-mail: bert.boyer@uaf.edu)

INSTITUTION: University of Alaska Fairbanks, Institute of Arctic Biology

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AWARD PERIOD: 1 June 2001 - 31 May 2004

REPORTING PERIOD: FINAL REPORT

SCIENTIFIC AND TECHNICAL OBJECTIVES: The overall objective of the proposed research has been to elucidate mechanisms of metabolic suppression using a hibernating ground squirrel model. Our initial objective was to focus on measuring proton leak associated with metabolic suppression during entrance into torpor in hibernating ground squirrels. Our second objective is based on our observation that poly A binding protein (PABP) is involved in mRNA stabilization during hibernation, and since PABP is involved in protein synthesis that consumes about a third of cellular energy, we set out to characterize the species specificity and molecular nature of PABP-protein and PABP-mRNA interactions. Lastly, our final objective has been to discover mRNAs that escape translational suppression and are up regulated during hibernation.

APPROACH: (1) To test the hypothesis that metabolic depression is regulated by changes in mitochondrial proton conductance, mitochondria were isolated from several tissues and proton leak was measured. (2) To identify the seasonal nature of PABP-mRNA binding, we have investigated the seasonal-dependency of PABP-mRNA interactions using explanted cells derived from liver of torpid and summer euthermic ground squirrels. (3) To identify mRNAs that may be upregulated and translatable during torpor, we used mouse developmental microarrays to identify genes whose mRNA is upregulated during torpor. More recently, we have also begun a comparative approach to understanding molecular mechanisms of metabolic suppression by preparing black bear cDNA libraries and sequencing them to develop a black bear array.

CONCISE ACCOMPLISHMENTS (200 words): We have completed specific aim I of our proposed research. We have published one of these manuscripts on proton leak and metabolic depression (Am J Physiol Regul Integr Comp Physiol. (2003) 284(5):R1306-13). We have submitted two additional manuscripts and both require revision before acceptance. One of these experiments was aimed at understanding the physiological significance of two candidate genes that may be involved in metabolic suppression (UCP 1 and UCP3). Our data suggests that UCP1 does serve a thermogenic function, but the associated increase in *Ucp3* expression does not play a role in thermogenesis. The third manuscript supports our hypothesis that leptin is involved in body mass regulation and *Ucp* homolog expression in hibernating ground squirrels. Finally, progress has been made in identifying several upregulated mRNAs in torpor using microarrays in ground squirrels and bears and two manuscripts describing this work are being written.

EXPANDED ACCOMPLISHMENTS (500 words): With our first specific aim completed, we turned our attention to specific aims II and III: To analyze the regulatory nature of PABP-mRNA binding by investigating the seasonal-dependency of these protein-mRNA interactions; and to identify translational control proteins bound to PABP and mRNA during torpor. To our complete surprise, the PABP-mRNA interactions were not specific to the hibernation season. These data strongly suggested to us that the PABP-mRNA interactions were simply a consequence of temperature. We therefore focused all of our attention to identification of additional genes upregulated during hibernation.

We used a three-phase approach. In the first phase, we examined the mRNA abundance of 9,600 genes in two states (torpid and summer active) from three target organs (liver, brown fat, and skeletal muscle) on a

mouse developmental cDNA array developed at the Wistar Institute. Several candidate upregulated mRNAs have been identified with the arrays have been analyzed through a combination of statistical and experimental analyses (quantitative PCR and northern blots). We have also prepared black bear cDNA libraries and have sequenced 7,000 unique clones that are being analyzed and prepared for an array study.

Much of our effort the past two years has been directed at statistical analysis of our microarray experiments and addressing the confounding effect of running squirrel RNA on mouse arrays. This is what has led us to develop a bear array for comparative studies. We have uncovered some interesting upregulated genes and are trying to verify their expression patters and potential function.

MAJOR ACCOMPLISHMENT BULLETS:

- We have discovered that mitochondrial proton conductance is unchanged during hibernation and that the reduced metabolism observed in hibernators is a partial consequence of tissue-specific depression of substrate oxidation.
- Using proton leak analyses we show that increases in *uncoupling protein 3* expression do not serve a thermogenic function. Rather, uncoupling protein 3 likely protects against the accumulation of fatty acids in the mitochondrial matrix.
- The polyA binding protein-mRNA interactions observed previously in hibernating animals are also observed in explants from squirrels not hibernating. These data suggest that PABP-mRNA interactions are a consequence of cold temperature.
- Using a mouse cDNA array, we have discovered several additional candidate genes that appear upregulated during hibernation. The functional significance of this result is currently being determined.
- We have developed black bear cDNA libraries for the ultimate construction of a bear array that will be used in future comparative studies of hibernation.

PUBLICATIONS, AWARDS AND PATENTS (during the project period):
PEER-REVIEWED JOURNAL PUBLICATIONS:

Barger JL, Brand MD, Barnes BM, Boyer BB. (2003) Tissue-specific depression of mitochondrial proton leak and substrate oxidation in hibernating arctic ground squirrels. Am J Physiol Regul Integr Comp Physiol. (2003) May;284(5):R1306-13

Shechter I, Dai P, Roseman MA, Gupta SD, Boyer BB, Guan G. (2003) Low temperature effect on the sterol-dependent processing of SREBPs and transcription of cholesterol and fatty acid related genes in HepG2 cells. J Lipid Res. 2003 May 16 [Epub ahead of print]

Additional manuscripts are in preparation.

BOOKS OR BOOK CHAPTERS: None

TECHNICAL REPORTS: None

PRESENTATIONS, POSTERS AND ABSTRACTS: DARPA meeting on hibernation. Breckenridge Colorado, September 2002.

JOURNAL AND ABSTRACT SUBMISSIONS: None

AWARDS, PATENT SUBMISSIONS, PATENTS ISSUED: None

TECHNOLOGY TRANSFER: Briefly describe what future plans you may have for technology transfer based on your ONR project. Technology transfer is an important measure of the relevance of scientific and technical endeavors. ONR Program Officers will use the information you provide here to highlight the technological payoffs that can emerge from investments in research.

The ultimate goal of our ONR funded research is to apply lessons learned from natural hibernators to related hypometabolic states in humans that are not currently well tolerated: trauma, stroke, and ressucitation. To transfer this technology, we first need to understand in detailed the molecular events specifying the hibernating phenotype in natual mammalian hibernators, and elucidate the critical components that fail when trying to re-create this phenotype in non-hibernating mammals.

ONR DATABASE STATISTICS:

Number of Papers Published in Refereed Journals Supported by ONR __2___

Number of Books or Chapters Published Supported by ONR__0___

Number of Technical Reports and Non-Refereed Papers Supported by ONR__0___

Number of Patents Issued: __0___

Number of Patents Pending: __0___

Number of Presentations: __0___

Number of Degrees Granted: _1____

Number of Principal Investigators (PI)/co-PI (Total): _1____
- PI/co-PI Women: __0___
- PI/co-PI Minority: _0____

Number of Associate Investigators (AI) (Total): _0____
- AI Women: __0___
- AI Minority: __0___

Provide names, addresses, phone numbers, fax numbers, and E-mail addresses of Co-PI and AI, if the information provided in your research proposal requires updating. __N/A___

Post Doctoral Students Supported (Total): ***___0__
- Post Doctoral Women: __0___
- Post Doctoral Minority: ***__0___

Number of Doctoral Students Supported (Total): ***__1 (research supply support)___
- Doctoral Women: __0___
- Doctoral Minority: **__0___

Number of Masters Students Supported (Total): ***__0___
- Masters Women: _0____
- Masters Minority: **__0___

Number of Undergraduate Students Supported (Total): ***__0___
- Undergraduate Women: __0___

- Undergraduate Minority: **__0___

** Under-represented or minority groups include Blacks, Hispanics, and Native Americans. Asians are not considered an under-represented or minority group in science and engineering.

*** Supported at least 25% this year on grant.

List Other Sponsored Work.
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None as PI.

List Foreign Collaborations (investigator, institution, location, objective).

None

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14. ABSTRACT

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